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Binding of leachable components of polymethyl methacrylate (PMMA) and peptide on modified SPR chip

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Abstract. Many types of polymers are often used in dentistry, which may cause allergic reaction, mainly methyl methacrylate allergy due to the leachable, degradable components of polymerized dental products. The aim of this study was to investigate the interaction between the leachable components of PMMA and peptides by Fourier-transform Surface Plasmon Resonance (FT SPR). In our previous work binding of oligopeptides (Ph.D.-7 and Ph.D.-12 Peptide Library Kit) was investigated to PMMA surface by phage display technique. It was found that oligopeptides bounded specifically to PMMA surface. The most common amino acids were leucine and proline inside the amino acids sequences of DNA of phages. The binding of haptens, as formaldehyde and methacrylic acid, to frequent amino acids was to investigate on the modified gold SPR chip. Self assembled monolayer (SAM) modified the surface of gold chip and ensured the specific binding between the haptens and amino acids. It was found that amino acids bounded to modified SPR gold and the haptens bounded to amino acids by creating multilayer on the chip surface. By the application of phage display and SPR modern bioanalytical methods the interaction between allergens and peptides can be investigated.

1. Introduction

Polymers have a major role in most areas of dentistry. Their properties allow a range of clinical applications not possible with other types of materials. The most common applications of polymers in dentistry include impression materials, aesthetic restorative materials, denture teeth, cements, dies, provisional crowns, endodontic fillings, tissue conditioners, pit, and fissure sealants. However, the primary use of polymers in terms of quantity is in the construction of complete dentures, the tissue-bearing portions of partial dentures, and the base-plates of removable orthodontic appliances. The polymethyl methacrylate (PMMA) denture base material is cured from methyl methacrylate (MMA) monomer by a free radical polymerization. This polymerization can be activated either by heating or chemically or both. The most often used initiators are benzoyl peroxide (BPO) as a heat activated and dimethyl-p-toluidine as chemical activated. The conversion of MMA monomers to PMMA polymer is not complete and some free monomer content remains in the polymer. The residual monomer release



from the polymer and can cause allergic reaction. The leachable components were analyzed by some researcher and it was found that the most frequent are (methyl) (meth)acrylate monomers, (meth)acrylic acid [1, 2], formaldehyde (FA) [3] and benzoin peroxide [4]. The leaching out process of components depends on many factors, like test conditions, medium, pH, thickness of inhibition layer on the surface, method of manipulation, powder-liquid ratio, curing process. The reactive functional group of methacrylate based materials is the unsaturated C-C bond, which is in not only the residual monomer but the formed polymer chain. This group is able to hydrolyze and oxidize. The hydrolysis product of functional group is methacrylic acid and the oxidation product is the formaldehyde. The cytotoxicity of denture base acrylic resin within methacrylate was the most investigated [5]. The formaldehyde as small molecules can cause allergy reaction in low concentration. The formaldehyde has crosslinked proteins, which leads to structural changing of peptide.

At the dental application of PMMA the residual free monomers together with other components leaching out from dental methacrylate based polymers and may contribute to local allergic reaction. Contact allergy is common among dental practitioners and patients. During the past two decades the incidence of allergies against dental materials has been rising. There is evidence that, while adverse reactions to dental materials are not as frequent, they can occur for many types of materials used in orthodontics, including alloys, resins, etc. Researchers found 2.3% positive patch test results to (meth)acrylate (2-hydroxyethyl methacrylate, HEMA; ethylene glycol dimethacrylate, EGDMA; bisphenol A diglycidyl ether methacrylate, bis-GMA) allergens in dental patients, and 5.8% of the dental personnel [6].

A lot of technologies have been used for quantified and qualified analysis of leachable molecules. High performance liquid chromatography (HPLC), gas chromatography (GC), mass spectrometry (MS) and capillary electrophoresis are the most often used technologies. Among these technologies the common is that the components are in dissolved form the polymer material. A lot of technologies have been developed that make it possible to survey large libraries of chemical structures, for example nucleic acids or peptides, for a target functional activity, such as binding to a receptor. Phage display is a powerful technique since it links peptide display with genetic information [7, 8]. The peptide display allows rapid selection, whereas the genetic information enables reliable amplification of phages, which results in the production of large amounts of potentially interesting targets. Phage display technology is used to investigate allergen-antibody interaction. In this study phage display technology is used to determine the amino acid sequence of binding phages on the PMMA denture base plate surface. Surface Plasmon Resonance spectroscopy (SPR) is a sensitive, modern bioanalytical real-time method for analyzing specific binding between different thin layers on the gold SPR chip [9, 10]. The interactions of allergens and peptides can be investigated by the application of phage display and SPR technique, which complexes are expressed in the molecular mechanism of allergic reaction.

The aim of our study was to investigate peptide structures bound to PMMA and interaction between them by SPR in order to deduct molecules may bind to PMMA and responsible for allergic reactions.

2. Materials and Methods

2.1. Preparation of PMMA sample

PMMA (Orthocryl, Dentaureum, Germany) samples were prepared according to the instructions of manufacturer. The parameters of samples were 3x3x1 mm. From the PMMA beads and surface fracture of polymerized product were taken Scanning Electron Microscope (SEM; Hitachi 3000N, Japan) pictures.

2.2. Phage display technique

For the Phage display technique Ph.D.-7 and 12 phage libraries (Biolabs, New England Peptide Library Kit) were applied. These libraries contained 7 and 12 members predefined sequences in DNA

of M13 coliphage. The PMMA samples were placed into eppendorf tube. The walls of tubes were blocked by bovine serum albumin (BSA) to ensure the direct binding of phages to PMMA surface. The phage solutions in PBS buffer was placed on the PMMA samples and stored at 37 °C. Biopannings were carried out New England Biolabs protocol. The bounded phages were amplified and determined the amino acid sequences with ABI PRISM BigDye Terminator Sequencing Ready Reaction Kit, Applied Biosystems (Warrington, United Kingdom).

2.3. Surface Plasmon Resonance Spectroscopy (SPR)

The Fourier-transform SPR spectroscope (FT SPR 100, Thermo Electron Corp., Waltham, MA, USA) equipped with Nicolet 6700 FT-IR (Thermo Electron Corp., Waltham, MA, USA) was used for investigate the interaction of amino acids and allergens. SPR is a sensitive, broadly applicable real-time method. FT SPR is performed a measuring of reflectivity over a series of angels of incident light of fixed wavelength. A minimum in reflectivity occurs at the “SPR angle” and at the “SPR wavenumber”, corresponding to the maximal SPR resonance. SPR reflectivity measurements are a surface sensitive spectroscopic method, which can be used to characterize the binding constant, thickness and refractive index of chemicals on metal surface.

2.4. Preparation of SAM layers, modification of SPR chip surface

The SPR chip surface was modified by using alkanethiol thus generated a self assembled monolayer (SAM) in favour of specific binding between the amino acids and allergens. Two different types of SAM layer were prepared on the gold surface according to Thermo Scientific Application note 51295. One is 6-amino-1-hexanethiol hydrochloride (AHT; Sigma-Aldrich) and the other one is 11-amino-1-undecanethiol hydrochloride (AUT; Sigma-Aldrich). 1mM solutions were prepared in ethanol from AHT and AUT. The gold chips were soaked in SAM solutions for 18 hours in dark. The thiol groups created covalent bonds with the gold, the amino functionalizes layer were formed on the chip surface. After the soaking the chips were washed first with large amounts of ethanol and then distilled de-ionized water. The chips were dried at room temperature and the chips have amine surfaces.

2.5. Preparation of amino acids and allergens solutions

The applied amino acids were leucine (Leu; Sigma-Aldrich) and proline (Pro; Sigma-Aldrich). The amino acids solutions were prepared in 1 mM concentration in PBS buffer at pH 7.4 (8.8 g NaCl; 0.24 g KCl; 3.11 g Na₂HPO₄ × 12 H₂O; 0.23 g KH₂PO₄). The investigated allergens were formaldehyde (FA; Sigma-Aldrich) and methacrylic acid (MA; Sigma-Aldrich) and the concentration of allergens solutions were 1 mM in PBS buffer.

3. Results and discussion

3.1. Preparation of PMMA

The SEM pictures were taken from the polymer beads and surface fracture of polymerized PMMA (Figure 1.). The SEM picture of the PMMA beads displayed the spherical, board size distribution polymers. The polymerized PMMA showed homogenous bulk material with swelled PMMA beads.

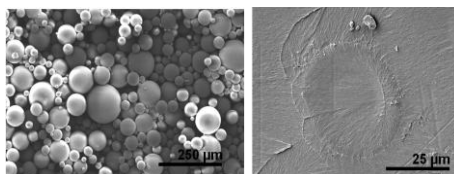


Figure 1. SEM picture of PMMA beads (left) and surface fracture of polymerized denture PMMA (right).

3.2. Result of Phage display technique

The results of the Phage display technique showed that the most frequent amino acids are leucine (Leu) and proline (Pro) in DNA sequences of specific binding phages to PMMA surface. Chemical structures of these amino acids are presented in Figure 2.

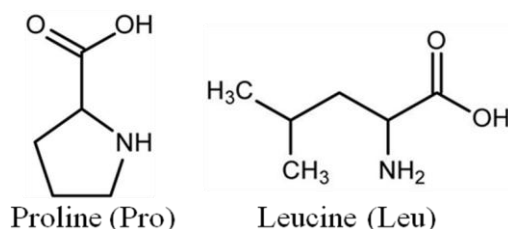


Figure 2. The most frequent amino acids in DNA sequences of specific binding phages.

3.3. Results of FT SPR measurements

3.3.1. Investigation of AHT-Leu-MA bindings

The modified SPR chip (AHT modified) was inserted into the SPR instruments, and the surface was washed by PBS buffer (Figure 3.). The background was taken to this measurements condition at 8964.77 cm^{-1} . Then the leucine solution was injected on the chip surface. The SPR sign (reflectance-wavenumber minimum) shifted toward lower wavenumbers value, indicating the binding of leucine to AHT layer. After that sensor chip and cell was washed intensively by PBS buffer. The position of SPR sign did not return to the position of the background, thus leucine bounded strongly to AHT layer. As a final step the methacrylic acid (MA) solution was injected into the cell creating a multilayer on SPR gold chip (Figure 4.). It was showed that the SPR sign shifted to lower wavenumbers value. The allergen is bounded to leucine, which was bounded to modified SPR chip because the MA is not washable by intensive PBS rinsing. Intermolecular hydrogen bonds were formed at 7.4 pH of PBS buffer between the AHT-Leu and Leu-MA.

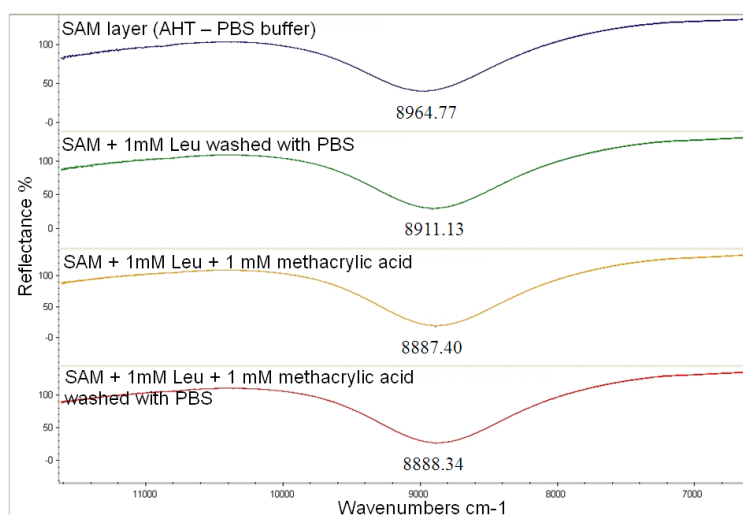


Figure 3. SPR measurements of AHT-Leu-MA bindings

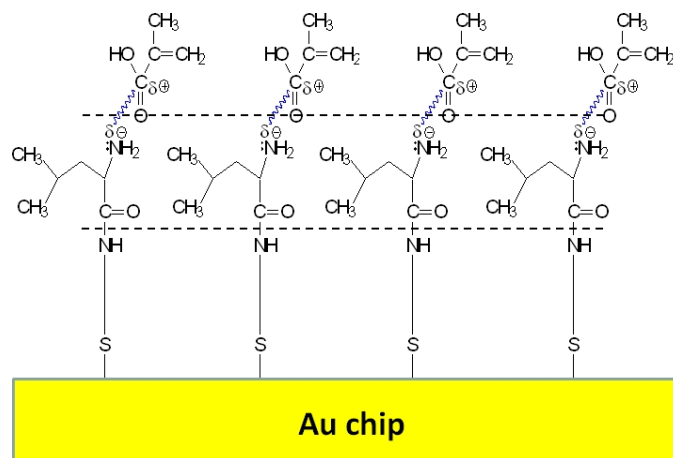


Figure 4. Multilayer on Au chip surface, Leucine-Methacrylic acid connection

3.3.2. Investigation of AUT-Pro-FA bindings

The SPR chip was modified by AUT for investigate proline-formaldehyde interaction (Pro-FA), Figure 5.). The surface was washed by PBS buffer (background), and then 1 mM proline solution was injected into the cell. The SPR sign shifted from 8989.19 cm⁻¹ to 8966.23 cm⁻¹ after the PBS washing. The proline amino acid bonded to modified SPR chip and proline remained on the surface after the PBS washing. In the next step formaldehyde solution was injected on the chip surface creating a multilayer on the chip surface (Figure 6.). The SPR sign shifted to lower wavenumbers (8946.71 cm⁻¹) indicating the binding of formaldehyde to proline. However the formaldehyde was washable from the surface because the SPR sign was closed to the value of AUT-Pro bindings after PBS rinsing. It was explained by the rigid molecular structure of proline, which hindered the formation of hydrogen bonds between Pro and FA.

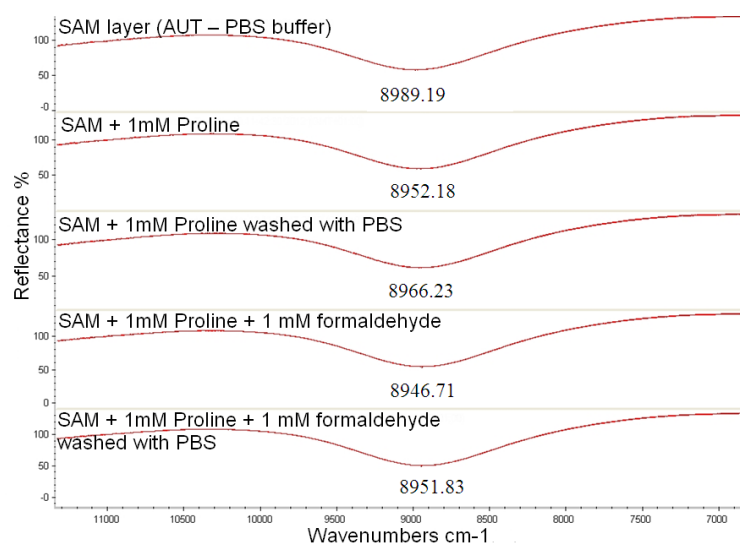


Figure 5. SPR measurements of AUT-Pro-FA bindings

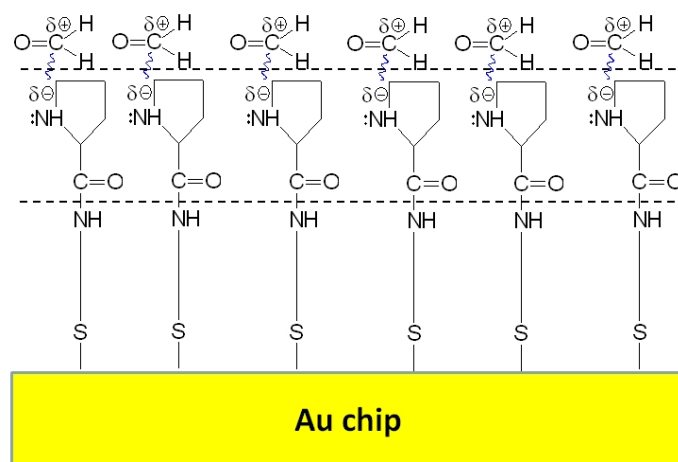


Figure 6. Multilayer on Au chip surface, Proline-Formaldehyde connection

4. Conclusion

The results of phage display and SPR measurements have shown that the most frequent amino acids are leucine and proline in the DNA of PMMA-specific phages. The bindings of two amino acids were investigated on modified SPR chip. It was found that the amino acids bounded to SAM layers despite of intensive PBS rising. At the injection of allergens solution the reflectance minimums shifted to lower wavenumbers value indicating the binding of MA and FA to amino acids. The binding of MA to Leu is stronger than FA to Pro on the chip surface. This is caused by the steric effect of Pro. The Pro has rigid structure due to heterocyclic ring, until the structure of Leu is more flexible. The intermolecular hydrogen bonding is formed more easily than in the case of proline.

5. Acknowledgments

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References

- [1] Yap A U J and Lee H K 2000 *Dental Materials* **16** 172
- [2] Koda T and Tsuchiya H 1990 *Dental Materials* **6** 13
- [3] Øysæd H and Ruyter I E 1988 *Journal of Dental Research* **67** 1289
- [4] Boeckler AF and Morton D 2008 *Dental Materials* **12** 1602
- [5] Jorge J H and Giampaolo E T 2003 *Journal of Prosthetic Dentistry* **90** 190
- [6] Goon A T and Isaksson M 2006 *Contact Dermatitis* **55** 219
- [7] Serizawa T and Sawada T 2005 *Journal of the American Chemical Society* **127** 13780
- [8] Serizawa T and Matsuno H 2011 *Journal of Materials Chemistry* **21** 10252
- [9] Date T and Tanaka K 2008 *Chemistry of Materials* **20** 4536
- [10] Matsuno H and Date T 2009 *Chemistry Letters* **38** 834